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A rapid method for detecting and mapping *in vitro* transcripts from supercoiled templates using endogenous RNase H

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Transcription of DNA templates in nuclear extracts is a widely used technique to study the sequences and factors involved in transcription by RNA polymerase II. Two types of templates are commonly used: linear templates, where a single product accumulates extending from the initiation site to the end of the template, and supercoiled templates. If the RNA is radiolabeled specific transcripts formed from linear templates can be detected directly by gel electrophoresis followed by autoradiography. Since RNA polymerase II does not terminate specifically there are not defined products formed by transcription of supercoiled templates by RNA polymerase II. Transcription from supercoiled templates can be assayed by either primer extension or nuclease protection assays, both of which require additional handling steps increasing the time required for the analysis and potentially introducing errors in recovery which can make quantitation more difficult. Since transcription from supercoiled templates is often more efficient than transcription from linear templates (1, and references therein) and is more likely to reflect the physiological situation, transcription from supercoiled templates is often the preferred assay.

Here we show that specific radiolabeled transcripts can be generated from a supercoiled template allowing direct measurement of transcription in a simple one-step assay. Inclusion of a DNA oligonucleotide complementary to the RNA transcript results in cleavage of all the transcripts from the supercoiled template at the same site by endogenous RNase H in the extract. RNase H cleaves RNAs in RNA–DNA hybrids (2,3) and is present in most nuclear extracts. Its activity has been utilized particularly in studies of RNA processing where specific small nuclear RNAs can be selectively destroyed (4–6) by appropriate antisense oligonucleotides. If a radioactive nucleotide triphosphate is included in the transcription reaction then RNase H will generate a single discrete radiolabeled RNA for each transcript from the supercoiled template. The length of the RNA transcript is determined by the position of the antisense oligonucleotide relative to the transcription start site.

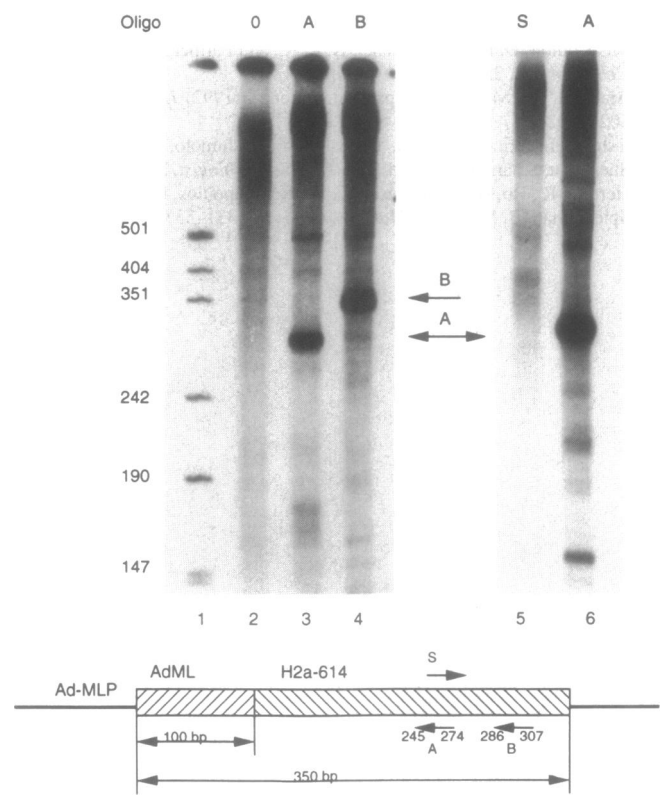

Figure 1. A supercoiled template containing the HLST gene was incubated in a nuclear extract in the presence of [^{32}P]UTP and the absence of exogenous oligonucleotides (lane 2) or in the presence of antisense oligonucleotides A (274–245, lanes 3 and 6), oligonucleotide B (307–286, lane 4) or the sense oligonucleotide S (lane 5). Lane 1 is pUC18 digested with *Hpa*II. The size of the DNA fragments are given. The RNAs migrate slower than DNA fragments of similar size. Below is a schematic of the HLST gene, which contains the adenovirus major late promoter (AD-MLP) followed by a transcribed region containing adenovirus and mouse histone H2a-614 sequence (hatched). The positions of the oligonucleotides A, B and S are shown.

Transcription in nuclear extracts. We constructed the HLST gene, which contains the adenovirus major late promoter fused to a portion of the mouse histone H2a-614 gene (Fig. 1). Nuclear extracts were prepared from mouse myeloma cells essentially by the method of Shapiro *et al.* (7). Transcription was carried out in a 20 µl reaction containing 1 µg template DNA, 15 µl nuclear extract, 7.5 mM $MgCl_2$, 1 mM ATP, CTP, GTP, 10 µM UTP and 10 µCi of [α-^{32}P]UTP (3000 Ci/mmol) and 80 mM KCl for 15

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or 30 min at 25°C. After addition of 1% SDS, 10 mM EDTA to stop the reaction, RNA was prepared by extraction with phenol and recovered by precipitation with ethanol. The RNA products were resolved by electrophoresis on 6% polyacrylamide–7M urea gels and detected by autoradiography.

Antisense or sense oligonucleotides to the region between 250 and 350 nt from the start site were synthesized and included in the reaction (0.5–1 μg per reaction). Thc antisense oligonucleotides will hybridize to the transcripts and direct cleavage of the transcripts by endogenous RNase H. Transcription from the supercoiled template resulted in a heterogeneous range of products (Fig. 1, lane 2). When an antisense nucleotide from 274 to 245 (Oligo A, Fig. 1, lanes 3 and 6) or from 307 to 286 (Oligo B, Fig. 1, lane 4) was included in the reaction, then a major specific product was detected of the expected size for transcripts initiated from the adenovirus major late promoter. Similar results were obtained with two other oligonucleotides located between 150 and 200 nt from the start of transcription (not shown), suggesting that the sequence or position of the oligonucleotide was not important. There were residual heterogeneous transcripts remaining, which could result from transcripts initiated from other sites in the template or from the 3′ portion of the cleaved transcripts. Inclusion of the sense oligonucleotide complementary to oligonucleotide A (Oligo S, Fig. 1, lane 5) did not alter the pattern or amount of the transcription products. All the transcription from this template in this extract was by RNA polymerase II, since transcription was blocked by addition of 2 μg/ml α-amanitin, which selectively inhibits RNA polymerase II (not shown). Treatment of the purified RNA products with additional RNase H in the presence of oligonucleotides did not alter the amount of cleaved transcripts (not shown). Thus, there was sufficient RNase H in the extract to cleave all the products.

This assay provides a rapid method for assessing transcription activity by RNA polymerase II on supercoiled templates and should be equally applicable for other RNA polymerases. There is minimal handling of the RNA product, which should make quantifying transcription activity more reliable. Inclusion of RNAse H in reactions in more purified systems (which may lack endogenous RNAse H) should make this assay applicable to those systems also.

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